Available online at www.sciencedirect.com
SCIENCE DIRECT®
Bioorganic & Medicinal Chemistry Letters
Bioorganic & Medicinal Chemistry Letters 15 (2005) 291–295

Detection of aptamer–protein interactions using QCM and electrochemical indicator methods

Tibor Hianik,[a,*] Veronika Ostatná,[a] Zuzana Zajacová,[a] Ekaterina Stoikova[b] and Gennady Evtugyn[b]

[a]*Faculty of Mathematics, Physics and Computer Sciences, Comenius University, 842 48 Bratislava, Slovakia*
[b]*Department of Chemistry, Kazan State University, 420008 Kazan, Russia*

Received 7 July 2004; revised 27 October 2004; accepted 28 October 2004
Available online 18 November 2004

Abstract—We report novel method of detection thrombin–aptamer interaction based on measurement the charge consumption from the electrode covered by DNA aptamers to an electrochemical indicator methylene blue (MB), that is bounded to a thrombin. The binding of thrombin to an aptamers has been detected also by QCM method in flow measuring cell. We showed that using MB it is possible to detect thrombin with high sensitivity and selectivity.

1. Introduction

DNA/RNA aptamers are single stranded nucleic acids with high affinity to proteins or to other low and macromolecular compounds, which is comparable with affinity of antibodies.[1,2] DNA/RNA aptamers can be chemically modified by thiol groups or by biotin, that allowing them to attach to the solid surface.[3] This system can be used as a biosensor for detection the thrombin,[4] antibodies[3] or other medically important molecules in complex biological liquids. RNA aptamers have been selected also for prions,[5] which open new possibilities for rapid diagnosis of transmissible spongiform encephalopaties (TSE). The detection of protein aptamer interaction has been performed by various methods, such are quartz crystal microbalance (QCM),[3] surface plasmon resonance (SPR)[6] or fluorescence method.[7] Considerable attention has been focused on detection the α-thrombin. This is connected with unique role of this serine protease in blood coagulation.[7,8]

In our recent paper we have shown that electrochemical indicator methylene blue (MB) could be used for the study of protein–DNA interactions.[9] It is known that MB is reduced to a leucomethylene blue (LB) at an electrode surface by accepting two electrons at certain reduction potential.[10,11] MB binds both to the DNA and to the proteins. This indicator can be therefore used also for detection of protein–aptamer interactions.

In this work we report novel method of detection thrombin–aptamer interaction based on measurement the charge consumption from the electrode covered by DNA aptamers to a MB, that is bounded to a thrombin. The binding of thrombin to an aptamers has been detected also by QCM method in flow measuring cell. We showed that using MB it is possible to detect thrombin with high sensitivity that is comparable with fluorescence detection method. The method based on MB is easier and do not require additional chemical modification of the DNA by fluorescence probe and by quencher as it does when aptamer beacons are used.[7]

2. Experimental section

2.1. DNA aptamer

We used 32-mer DNA aptamer modified by biotin at 3′ end of the following sequence: 3′-BIO-GGG TTT TCA CTT TTG TGG GTT GGA CGG GAT GG-5′. This aptamer has at its 5′ end typical motif with high affinity to the thrombin.[12] The aptamer has been synthesized by Generi Biotech, Czech Republic and used as obtained.

Keywords: Aptamer; Thrombin; Methylene blue; Cyclic voltammetry; Charge consumption.
* Corresponding author. Tel.: +421 2 60295683; fax: +421 2 65426774; e-mail: hianik@fmph.uniba.sk

0960-894X/$ - see front matter © 2004 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmcl.2004.10.083